

Claims

1. Use of a culture medium comprising
 - at least one meiosis activating sterol (MAS), a MAS being any sterol in the metabolic pathway between lanosterol and cholesterol,
- 5 - a MAS analogue, or
 - an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS for *in vitro* fertilisation, the from the *in vitro* fertilisation resulting pre-embryo having an improved implantation rate *in vivo*.
- 10 2. Use of at least one MAS, a MAS analogue, or an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS in cumulus enclosed oocytes for the preparation of a culture medium for *in vitro* fertilisation, the from the *in vitro* fertilisation resulting pre-embryo having an improved implantation rate *in vivo*.
- 15 3. Use according to claim 1 or 2, wherein MAS is selected from the group consisting of FF-MAS, T-MAS, 1-methyl-zymosterol, and zymosterol.
4. Use according to any of the preceding claims, wherein MAS is FF-MAS.
- 20 5. Use according to any of the preceding claims, wherein MAS is T-MAS.
6. Use according to claims 1 or 2, wherein the culture medium comprises a MAS analogue.
- 25 7. Use according to claims 1 or 2, wherein the additive or additives leads to a ratio of at least 2 between the relative content of MAS in cumulus enclosed oocytes cultured in the presence of the additive or additives, the relative content of MAS in cultured cumulus enclosed oocytes being determined by stimulation female mice with exogenous gonadotropins 48h prior to removal of the ovaries from the mice and recovering cumulus enclosed oocytes from the ovaries by puncturing individual follicles and culturing the
- 30 recovered cumulus enclosed oocytes in an α -MEM medium supplemented with 3mg/l bovine serum albumin, 5 mg/l human serum albumin, 2mM L-glutamin, 100IU/ml penicillin, 100 μ g/ml streptomycin, 4mM hypoxanthine and 3 H-mevalonat for 24h at 37°C, 100% humidity and 5% CO₂ in air, followed acidification with 50 μ l 0.3M Na₂PO₄ pH=1,

organic extraction three times with a five-fold surplus of n-heptane:isopropanol (3:1 v/v), purification of MAS from the organic phase by HPLC and determination of the ratio of radioactivity per cumulus enclosed oocyte between cumulus enclosed oocytes cultured in the presence of the additive or additives and cumulus enclosed oocytes cultured without
5 the presence of the additive or additives.

8. Use according to claim 7, wherein the additive is selected from the group consisting of gonadotropins such as FSH and analogues, growth hormones such as EGF and analogues, compounds inhibiting sterol Δ^{14} -reductase such as AY9944-A-7, compounds
10 inhibiting 4-demethylase converting T-MAS to Zymosterol, compounds activating cytochrome P450 lanosterol 14α -demethylase and compounds with an amphotericin like effect.

9. Use according to claim 8, wherein the additive is a combination of a gonadotropin and a
15 growth hormone.

10. Use according to claim 9, wherein the additive is a combination of EGF and FSH.

11. Use according to claim 8, wherein the additive is EGF.
20

12. Use according to claim 8, wherein the additive is FSH.

13. Use according to claim 10 or 12, wherein FSH is an FSH isoform with an isoelectric point above 5.0.
25

14. Use according to any of claims 10, 12 or 13 wherein the FSH is derived from naturally occurring FSH such as FSH extracted from urine, or from recombinant FSH.

15. Use according to any of claims 10 or 12-14, wherein the concentration of FSH is
30 between 2 and 200IU FSH/l.

16. Use according to any of claims 10 or 11, wherein the concentration of EGF is between 1 and 10ng EGF/ml.

35 17. Use according to claims 7 or 8, wherein the additive is amphotericin.

18. Use according to any of the preceding claims, wherein the *in vitro* fertilisation is *in vitro* fertilisation of human oocytes.

- 5 19. A method for *in vitro* fertilisation comprising the step of: exposing and culturing one or more MII oocytes with spermatozoa in a culture medium, the culture medium comprising at least one MAS, a MAS analogue, or an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS; the exposure and culturing lasting until zygotes and/or pre-embryos are formed.

10

20. A method for *in vitro* fertilisation comprising the steps of:

(a) culturing one or more GV oocytes in a culture medium, the culture medium comprising at least one MAS, a MAS analogue, or an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS; hereby forming one or more MII

15 oocytes;

(b) exposing and culturing the one or more MII oocytes of step (a) with spermatozoa in a culture medium, the culture medium comprising at least one MAS, a MAS analogue, or an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS; the exposure and culturing lasting until zygotes and/or pre-embryos

20 are formed.

21. A method according to claims 19 or 20, wherein the MII oocytes are cumulus enclosed oocytes.

- 25 22. A method according to any of claims 19-21, wherein the culture medium comprises an additive or additives capable of endogenous stimulation of the accumulation of at least one MAS.

23. A method according to any of claims 19-21, wherein the culture medium comprises

30 FF-MAS, T-MAS, 1-methyl-zymosterol, and/or zymosterol.

24. A method according to any of claims 19-21, wherein MAS is FF-MAS.

25. A method according to any of claims 19-21, wherein MAS is T-MAS.

35

26. A method according to any of claims 19-21, wherein the culture medium comprises a MAS analogue.

27. A method according to any of claims 19-26, wherein the oocytes are nude.

5

28. A method according to any of claims 19-27, wherein the culture medium comprises at least one MAS or at least one MAS analogue.

29. A method according to any of claims 19-18, wherein 1-15 oocytes are cultured and
10 exposed together.